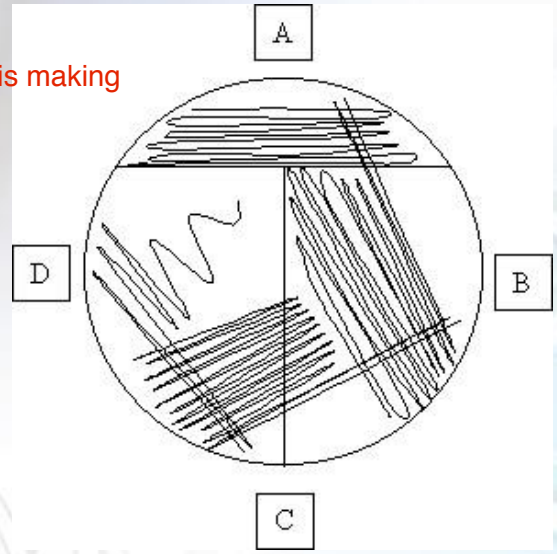
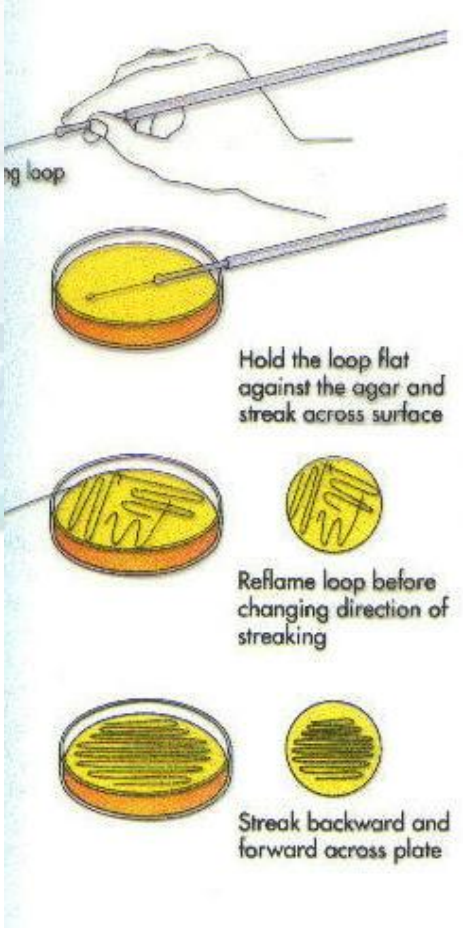
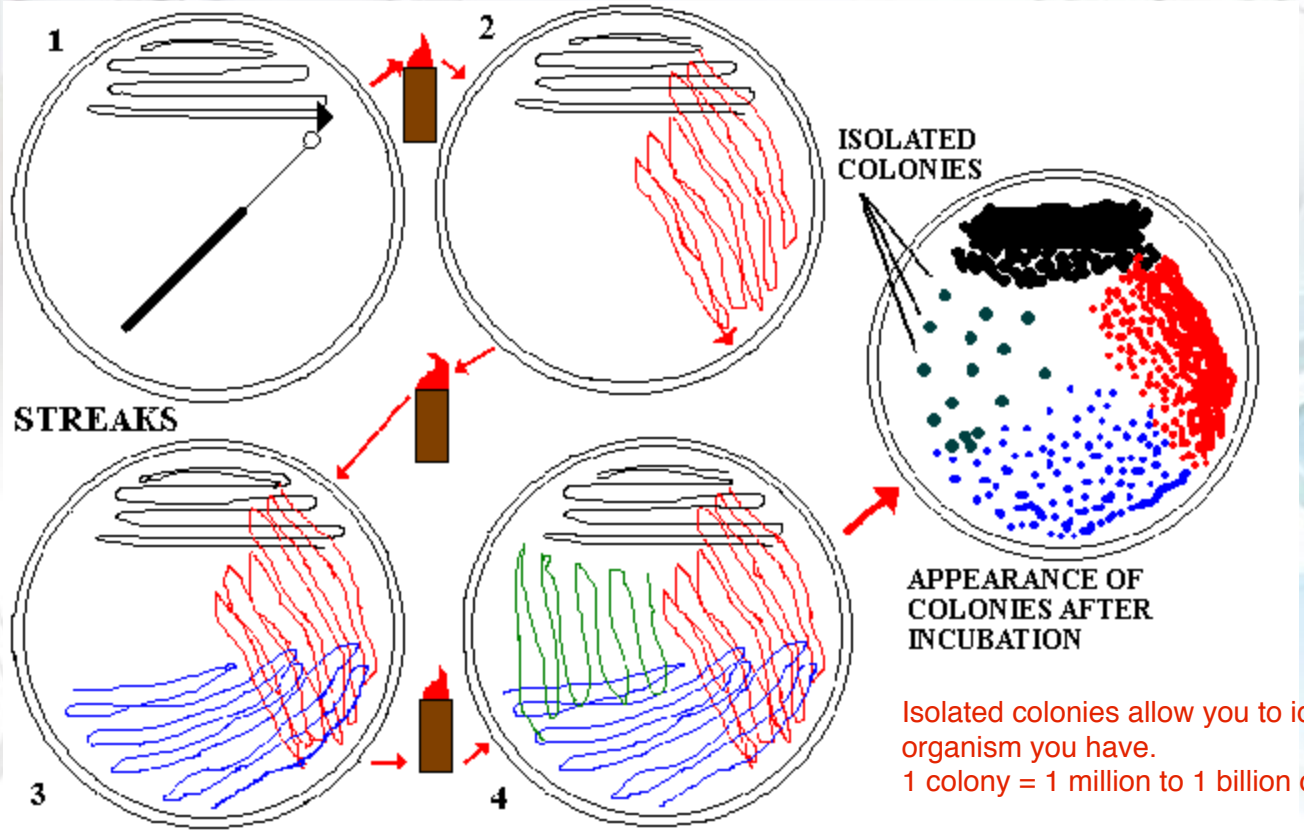
Diagnostic Microbiology

- Isolation of pure culture from specimen
  - ✓ why?
    For identification (figure out what is making you sick)
- Culture media
  - ✓ why?
  - ✓ what?
  - ✓ who?
- Inoculation methods
  - ✓ streak, spread, or pour



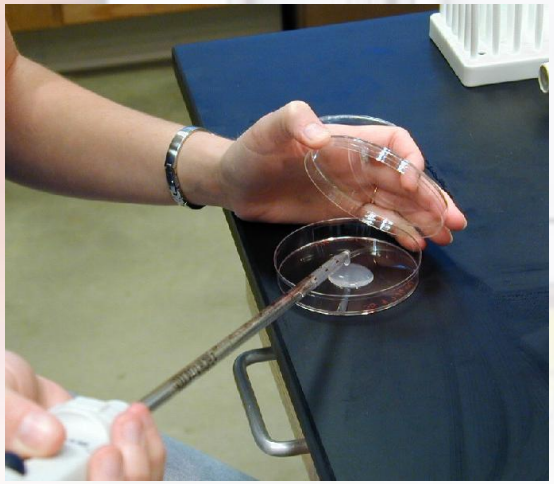


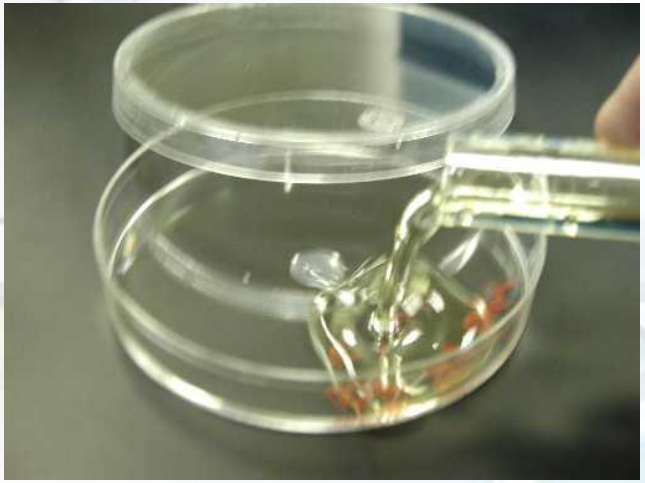
Streaking a plate for isolated colonies



Isolated colonies allow you to identify what organism you have.
1 colony = 1 million to 1 billion cells

Pour plates





- Bacteria grow inside agar
- Used for enumeration of bacteria

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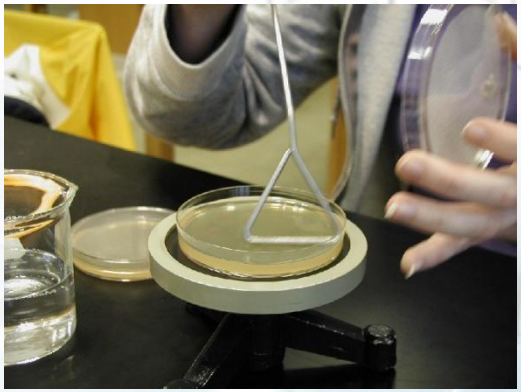
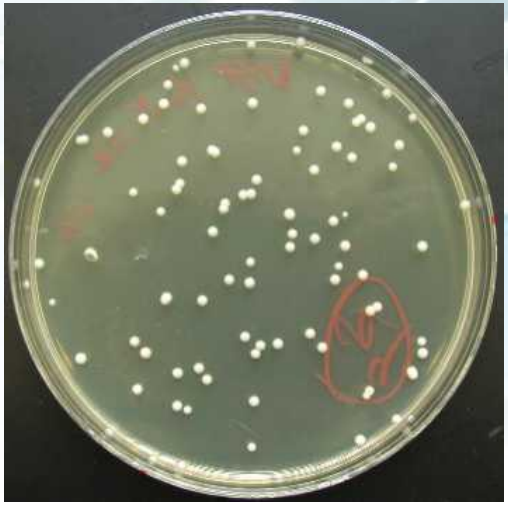
Spread plates





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Preservation of Cultures

- pure cultures of bacteria are stored:
- freeze-dried (lyophilized)
- frozen at -80C

Becomes powder, you can then grow it back whenever you want it

lyophilizer

Ultra-low freezer

Preservation of pure cultures

- Why would we want to keep a “copy” of a bacteria we isolated from a patient?

  To study how the bacteria evolved, why we’re having problems in certain areas (ex. ontario but not quebec)

- Short term versus long-term

  - ✓ liquid nitrogen (-195^{o}C)
  - ✓ freezers
  - ✓ lyophilization (freeze-drying)

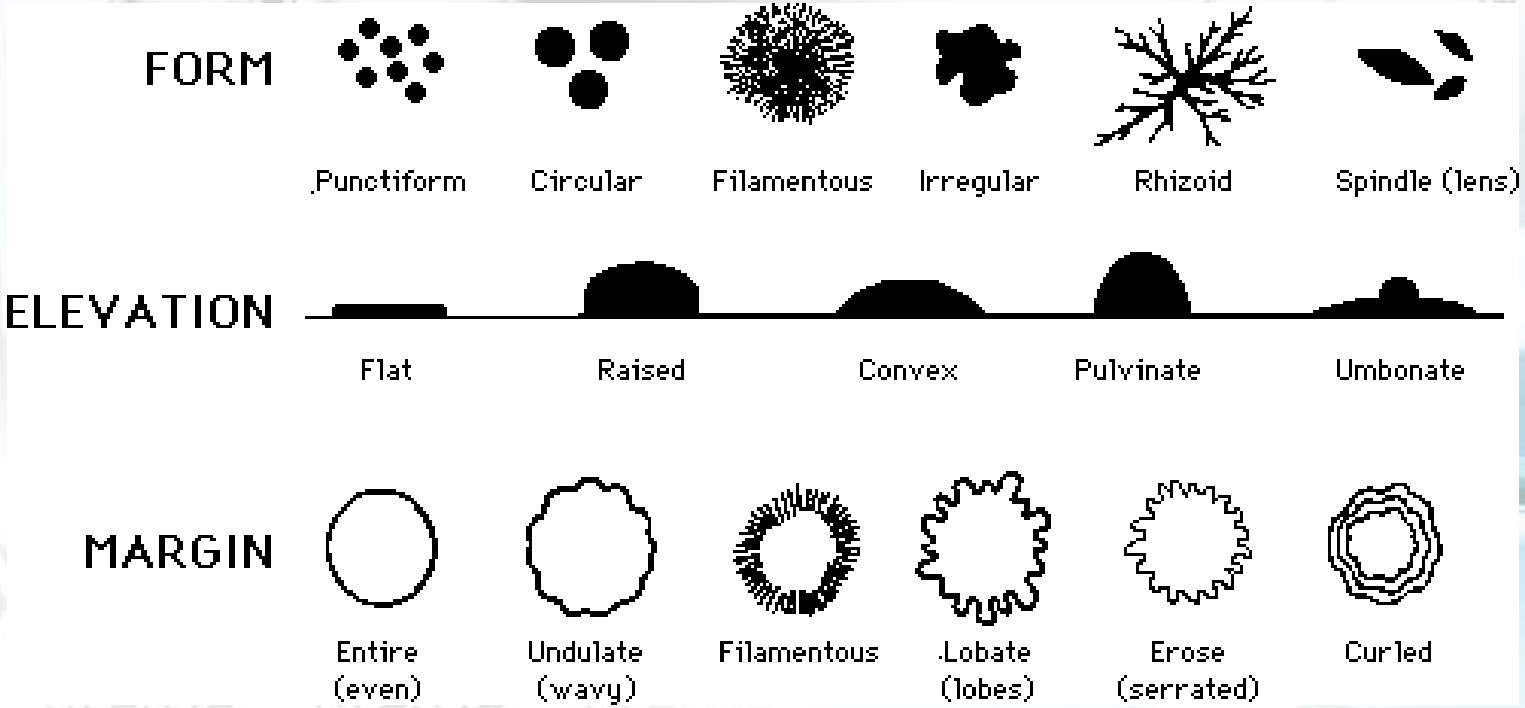
Identification

- Now that you have a pure culture…
  - ✓ colony morphology
  - ✓ cellular morphology

- The microscope is your friend
  - ✓ resolving power (resolution) = ability to distinguish two closely located objects as separate, distinct entities

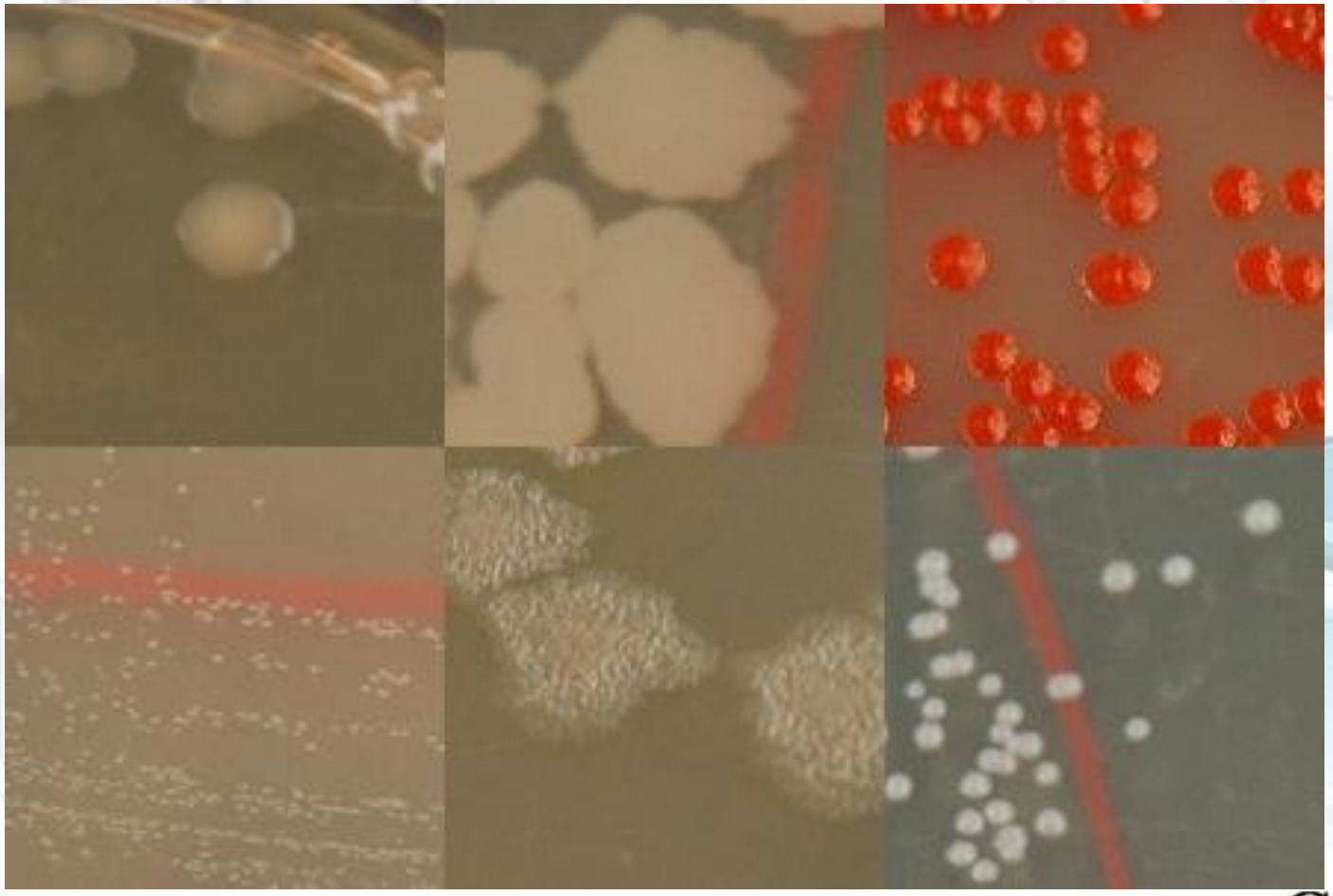
Colonial morphology

The two top parts of the “Y” molecule are the parts that bind to the antigen, the bottom part of the “Y” can be tagged (for example with a florescent colour to make them visible) - this was said about a different picture that we don’t have



DO NOT HAVE TO REMEMBER THESE

Examples of colonial morphologies



Identification

- Staining Techniques -

- Generally, three steps:

1. Make a smear
2. Fix dried smear by heat
3. Stain with desired dye

"Y" tails coloured?

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Simple vs. Differential staining

- Simple stain
  - ✓ single dye normally used
  - ✓ all organisms same colour
  - ✓ size, shape, number, arrangement, etc.

If you do not see anything on the slide, there's no bacteria and the illness is probably a viral infection

- Differential stain
  - ✓ two or more dyes
  - ✓ differences between microorganisms or parts of cells
  - ✓ acid fast, **Gram**

The Gram Stain

(Hans Christian Gram)

Know this well!

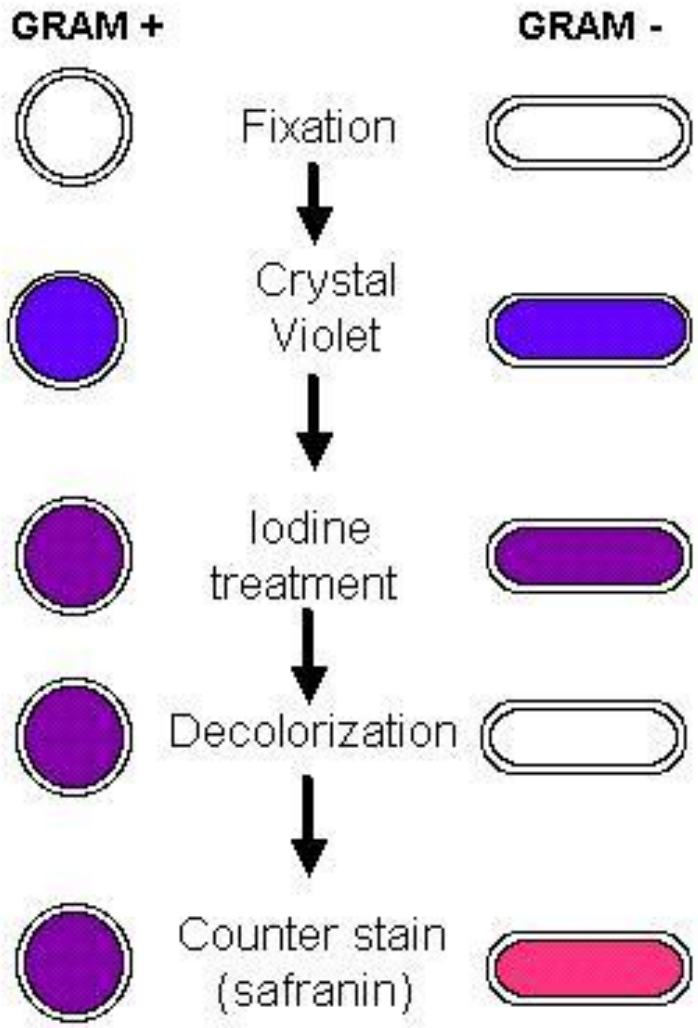
1. **Flood slide with crystal (or gentian) violet. (Wash with running tap water).**
2. **Flood with Gram's iodine. (Wash with water).**
3. **Carefully decolorize with 95% ethanol. (Wash with water).** Has to be timed for the staining to work

This third step is the most critical and also the one most affected by technical variations in timing and reagents.

Gram - becomes invisible here so it must be coloured

4. **Flood with safranin (pink color). (Wash with water). Air dry, or blot with absorbent paper .**

Until you've done the counter stain, you don't know whats on your slide. You have to do the counter stain to see if you have gram negative bacteria.



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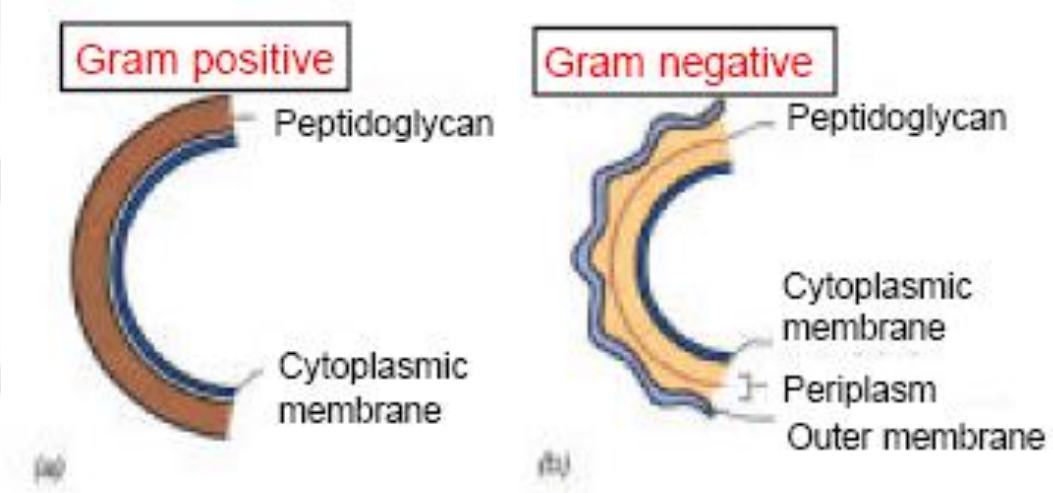
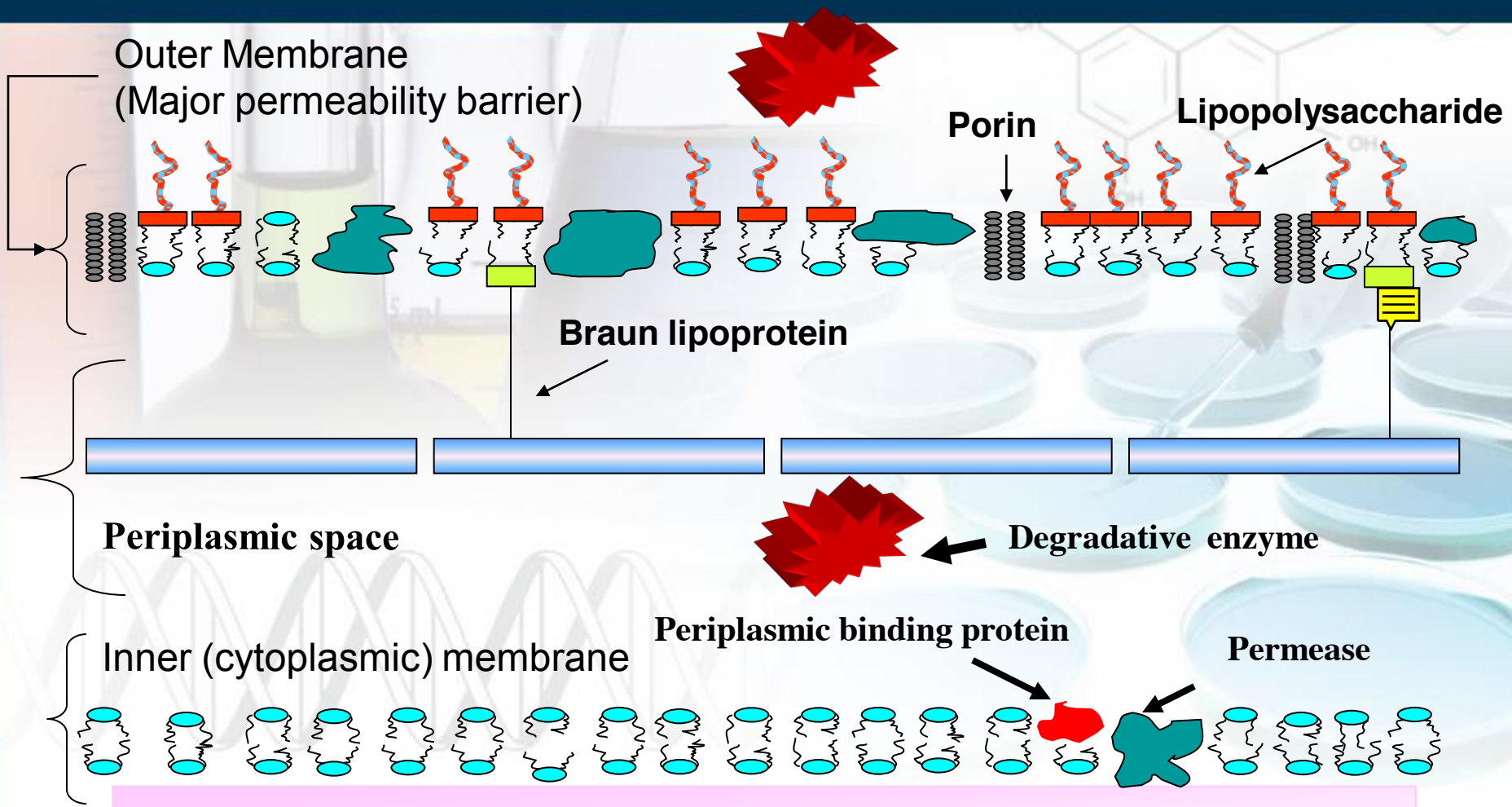
Cell wall is the key!

- Essential for cell growth and division
- Shape of bacteria related to peptidoglycan layer
- Gram negative usually thinner than Gram positive

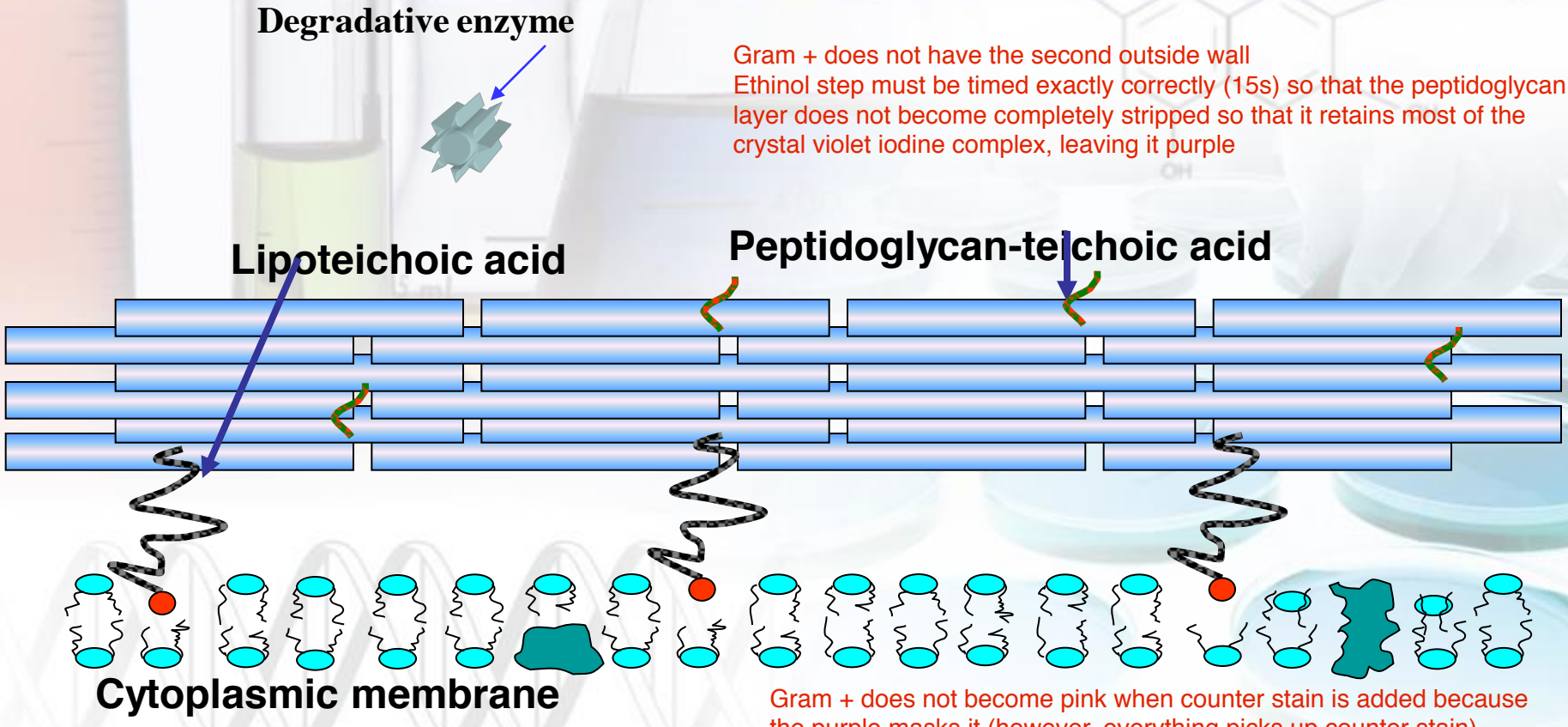


Reason why Gram - goes invisible during washing

GRAM NEGATIVE CELL ENVELOPE



GRAM POSITIVE CELL ENVELOPE



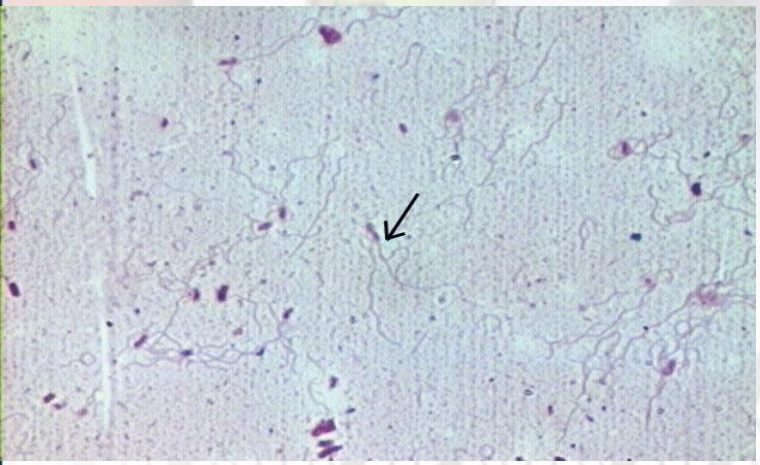
Gram + does not have the second outside wall
Ethinol step must be timed exactly correctly (15s) so that the peptidoglycan layer does not become completely stripped so that it retains most of the crystal violet iodine complex, leaving it purple

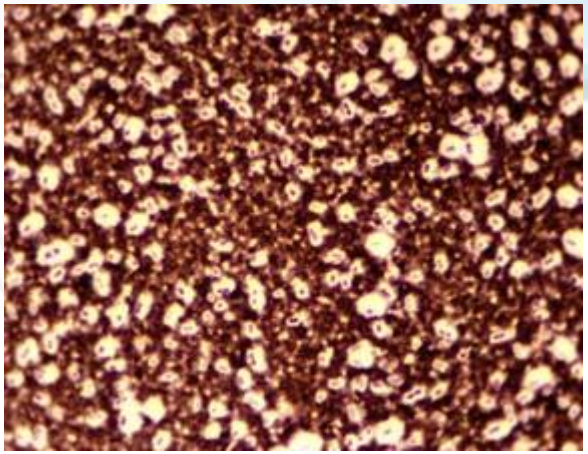
Gram + does not become pink when counter stain is added because the purple masks it (however, everything picks up counter stain)

Cytoplasm

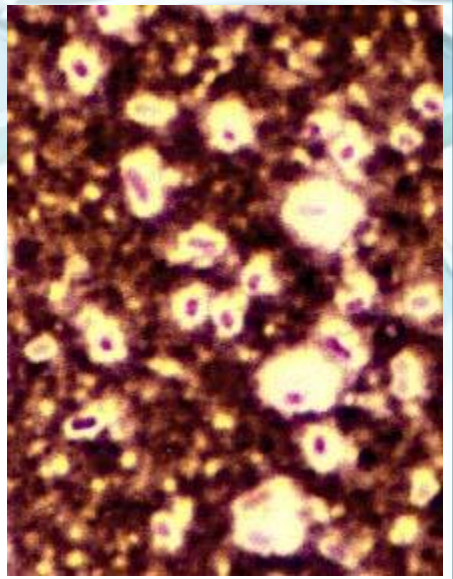
Other stains

- Endospore
- Capsule
- Flagella



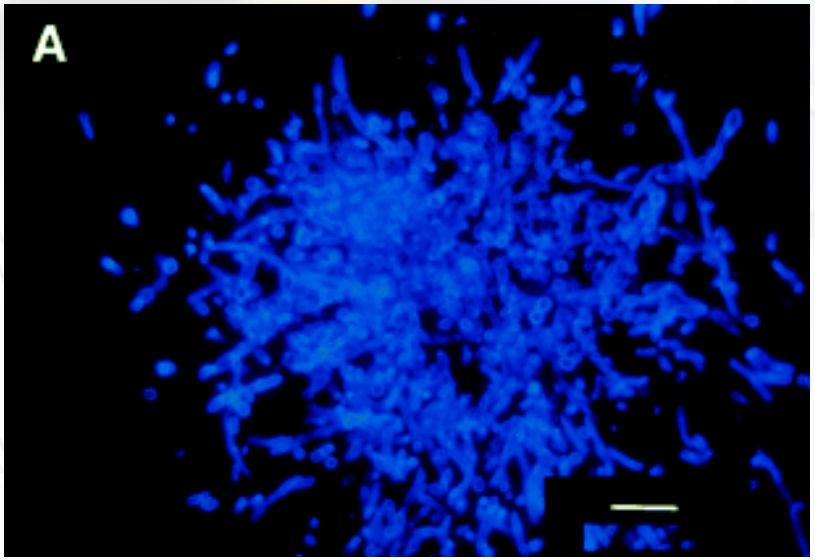


Capsules



Fluorescence microscopy

- dye fluoresces at specific wavelength
- antibodies tagged with dyes are common (immunofluorescence microscopy)

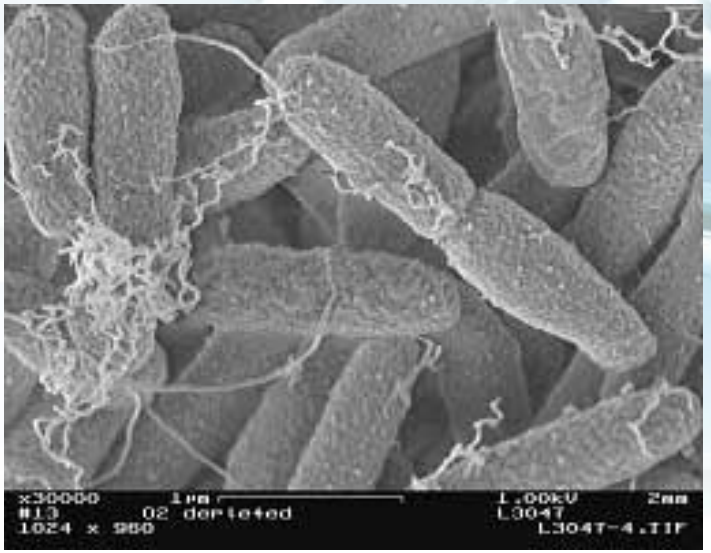


A section of the liver of a leukemic patient who had succumbed to culture-proven *C. albicans* mycosis. (staining done with Blankophor) - keep your mind open and ask proper questions

Antibodies with the bottoms stained

Electron microscopy

- Electron beam (instead of light)
- Million times magnification possible (0.003 µm)
  - ✓ TEM (stain with heavy metals)
  - ✓ SEM (3-D image of cell surface)



Electron microscopy

Scanning electron microscope (SEM)

Transmission electron microscope (TEM)

So what's the bottom line?

- Morphology helps to classify and identify
  - ✓ Gram stain
- Gives clues to how they behave in environment
  - ✓ capsules, endospores

The big thing staining is useful for is to be able to rule out bacterial infection (if there is nothing on the slide it is not a bacterial or parasitic infection)

Characteristics of bacteria

- Small (0.75 – 1.25 µm in diameter/width)
- Higher surface area / volume ratio
  - ✓ higher metabolism
  - ✓ faster growth
  - ✓ replication rate (~20 minutes)

Shapes and sizes of bacteria

- Bacteria are usually arranged in specific patterns:
  - ✓ single cells (spiral and/or rod shaped)
  - ✓ diplococci (pairs) – single plane
  - ✓ chain (divide in one plane and remain attached)
  - ✓ tetrads (cocci dividing at right angle to first plane of division)
  - ✓ division in three planes (grapelike clusters)
  - ✓ cubical packet of 8 cells (sarcinae)

Don't necessarily have to remember this, just know that the appearance of what you see is very important

Shapes and sizes of bacteria

The Rod-Shaped Bacteria

Single bacillus

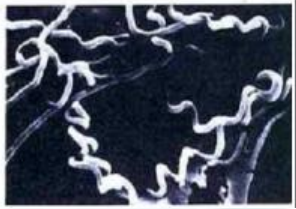
Diplobacillus

EXAMPLES: *E. coli, Yersinia pestis, Pseudomonas aeruginosa*

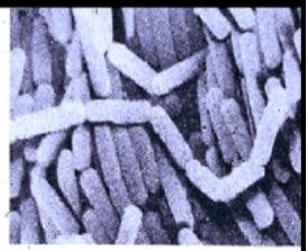
Vibrio

Spirillum

Spirochete



Streptobacillus

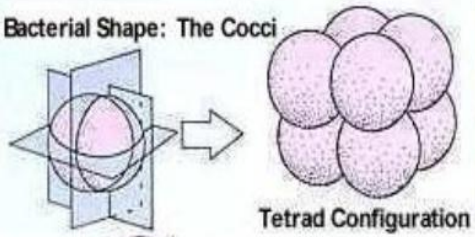


EX: *Haemophilus influenza*

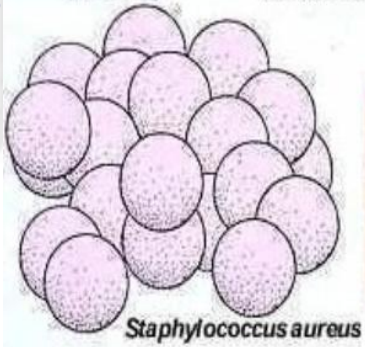
Coccobacillus

!!!These are only examples of general shapes!!!

Bacterial Shape: The Cocci



Tetrad Configuration



Staphylococcus aureus

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Some bacteria are visible to the naked eye!!!

With what do we grow bacteria?

Salmonella typhimurium

Gram Negative: growth

Lactose Fermentation: negative (colorless colonies)

Escherichia coli

Gram Negative: growth

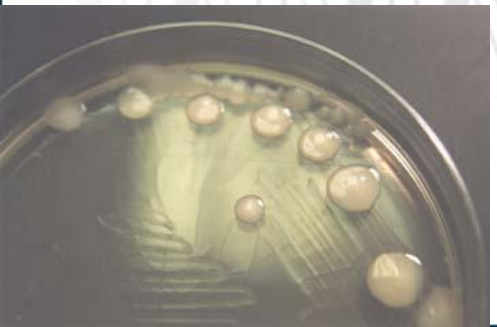
Lactose Fermentation: positive (pink colonies)

Definitions….☺

- Chemically defined – exact composition known
- Chemically undefined – some components can't be controlled (beef extract, blood, etc.)
  Classic example = blood agar plate
- If solid (versus liquid) growth – 1.5% agar used
  (can be solid or liquid)
- Enrichment media – increase # of specific bacteria in sample by favouring growth of interested species
  We want to grow the sample to a large concentration to be able to do stuff with it
- Tissue culture media – for cultivating viruses, derived of plant or animal cells

General media requirements

- Bacteria – requirements vary
- Yeasts – high sugar and lower pH
- Anaerobes – must remove oxygen



Selective, differential and S/D media

- Selective media – enhance growth of one bacterial species or suppression of another

  Really important because the type of antibiotic regiment you use is determined by the gram stain

- Differential media – differentiate bacteria based on their nutritional requirements and phenotypic characteristics

- Selective / Differential media – very useful in clinical labs (e.g., MacConkey agar) —> Most of the time they're both.

Ability and inability to ferment different sugars ex. ability to utilize lactose sugars

MacConkey – S/D media

(Bile salts, crystal violet inhibit Gram +ves)

Don't have to remember all agars but remember this one

Salmonella typhimurium

Gram Negative: growth

Lactose Fermentation: negative (colorless colonies)

Escherichia coli

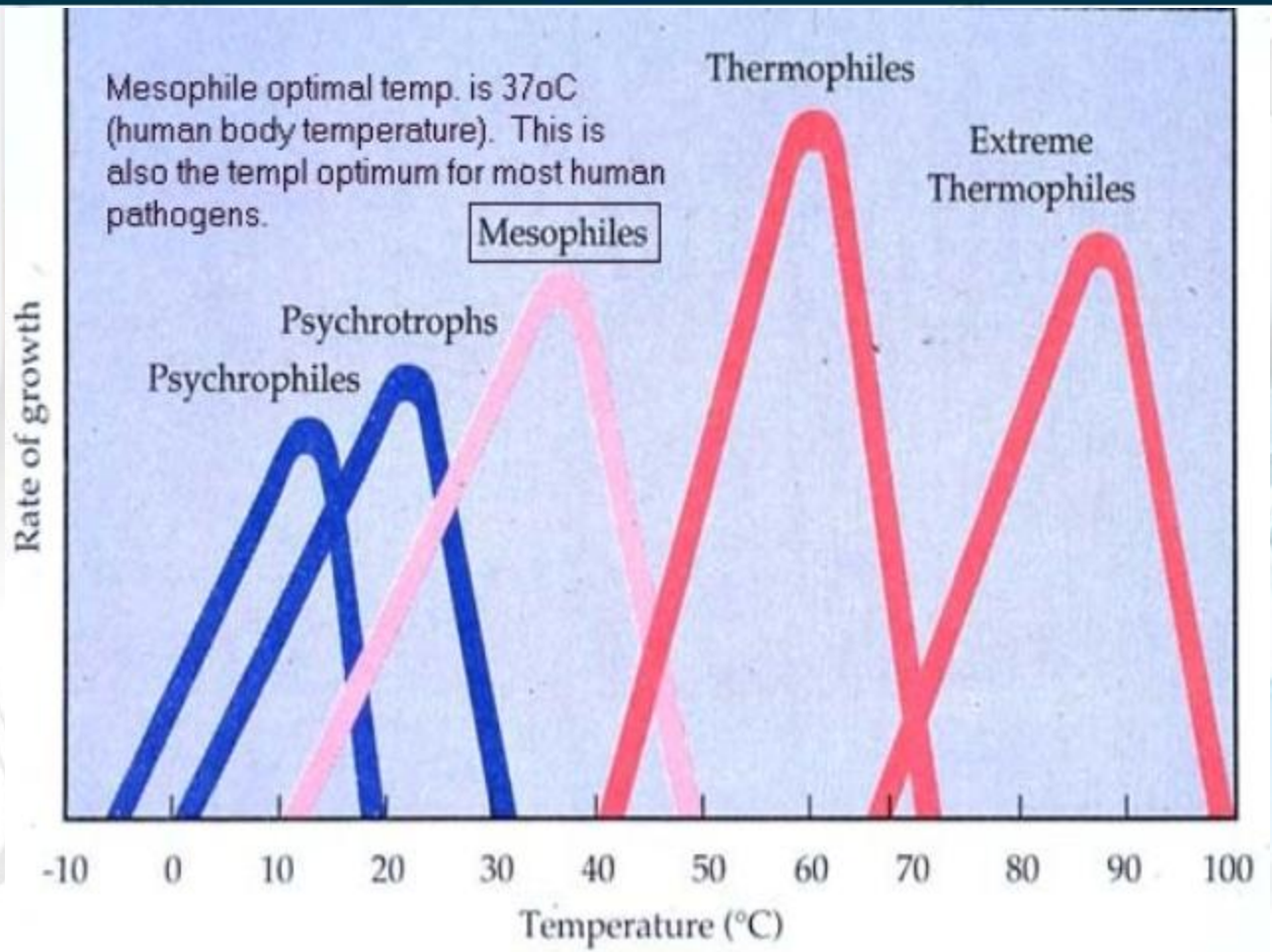
Gram Negative: growth

Lactose Fermentation: positive (pink colonies)

You can tell salmonella from E.coli based on colour

Know the word bacterium !! It means bacteria in the urine

Temperature requirements



These words are important (they mean they like these specific tempuratures)
Mesophiles are the most clinically relevant

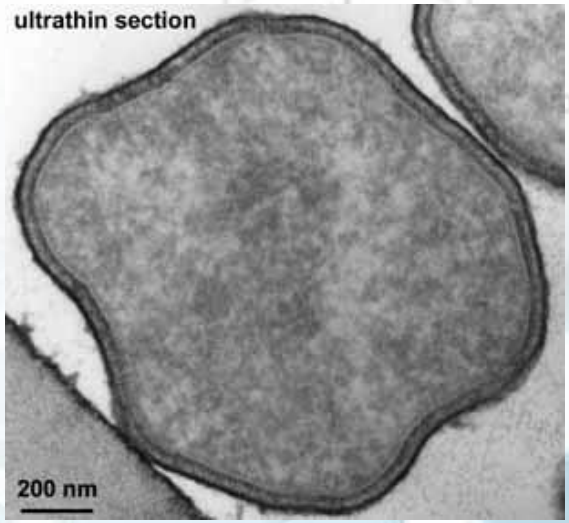
Wisteria is psychotrophic

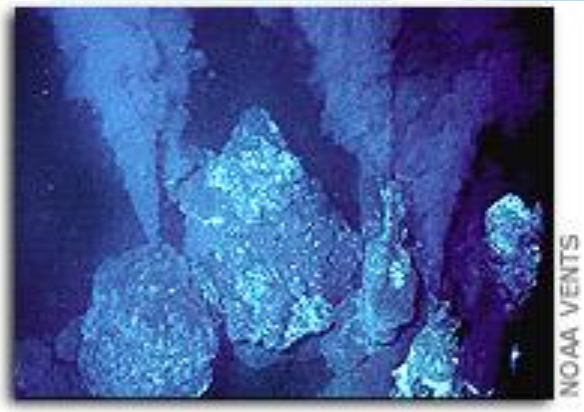
Temperature definitions

- Psychrophiles
  - ✓ grow best at temperatures 15-20°C
- Mesophiles
  - ✓ grow best at temperatures 25-40°C
  - ✓ most bacteria belong here
- Thermophiles
  - ✓ grow best at temperatures 40-85°C

Extreme Thermophiles

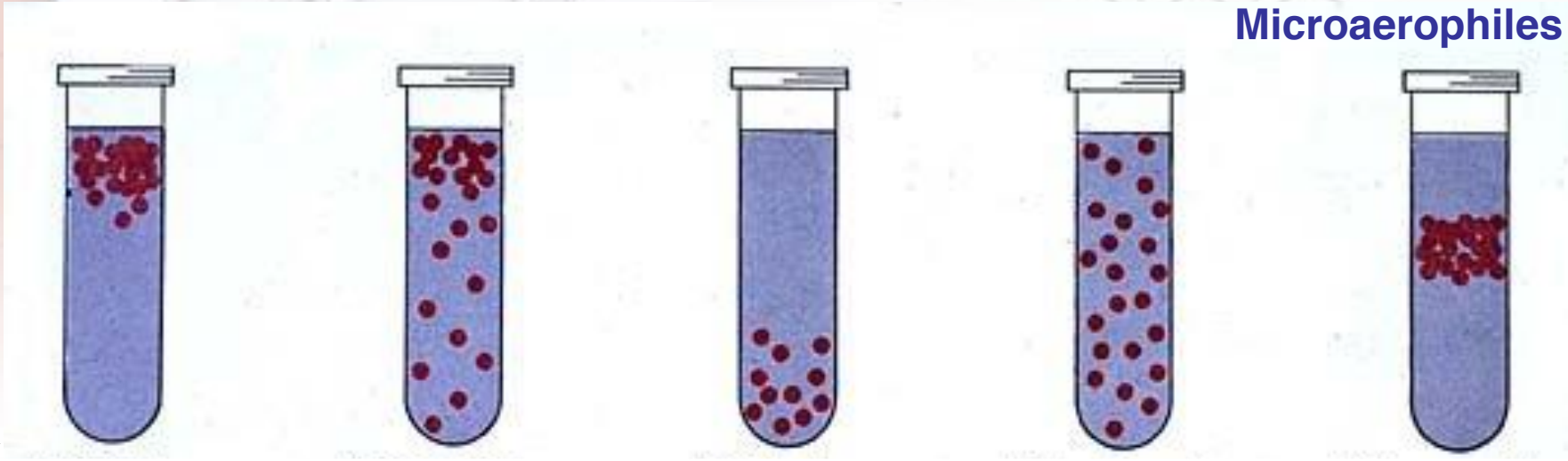
- *Pyrolobus fumarii*
- “fire lobe of the chimney”
- Lobed shape
- Discovered in the walls of a deep sea hydrothermal vent
- Grows between 30 and 113C
    - 106C is optimal





Oxygen requirements

Decreasing oxygen concentration



Obligate aerobes

Facultative aerobes

Obligate anaerobes

Aerotolerent anaerobes

Microaerophiles

Too much and too little oxygen are no good (they need a specific amount)

Incubators must have very strict temps and very strict atmospheres

Growth of anaerobic bacteria

Anaerobic jar

Coy anaerobe chamber

Indicator strip tells you that oxygen is getting in

* heat fix is to denature some proteins so bacteria will stick to the slide

pH and Water requirements

- Optimal pH varies from bacteria to bacteria
- Intracellular pH must be ~7.5
- Growth observed at pH values of 4-9 (optimum 6-8)

- Water (light) can be important for certain microorganisms

- Osmotic pressure (hypertonic, hypotonic, isotonic)

More important but tend to be forgotten

ELISA

Direct ELISA looks for the antigen in the sample
You place an antibody that looks for the specific virus
If you do have the virus, the antibody will bind to the antigen
Add antivirus antibody that will bind to the antigen (bound to the antibody)

Indirect ELISA detects immune response (how much antibodies you have made)
Places the antigen in the body to look for the antibody (to see if you have been exposed to the virus and built up immunity to it)

Home pregnancy test is a direct ELISA test that detects the pregnancy hormone in the urine which is the antigen
Steroids, chemicals and drug tests are also direct ELISA tests (they are looking for the drugs or hormones which are the antigens)